Effects of *CCR5-Δ32* and *CCR2-64I* alleles on HIV-1 disease progression: the protection varies with duration of infection

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Objective: To examine temporal variation in the effects of *CCR5-Δ32* and *CCR2-64I* chemokine receptor gene polymorphisms on HIV-1 disease progression.

Design: Pooled analysis of individual patient data from 10 cohorts of HIV-1 seroconverters from the United States, Europe, and Australia.

Methods: We studied HIV-1 seroconverters of European (n = 1635) or African (n = 215) ancestry who had been genotyped for *CCR5-Δ32* and *CCR2-64I*. We used Cox proportional hazards models with time-varying coefficients to determine whether the genetic protection against AIDS (1987 case definition) and death varied with time since seroconversion.

Results: Protection against AIDS conferred by *CCR5-Δ32* held constant at a 31% (RH 0.69, 95% CI 0.54, 0.88) reduction in risk over the course of HIV-1 infection, whereas protection against death held constant at a 39% reduction in risk (RH 0.61, 95% CI 0.45, 0.88). When the period from AIDS to death was isolated, the survival benefit of *CCR5-Δ32* diminished 2 years after AIDS. Protection against AIDS conferred by *CCR2-64I* was greatest early in the disease course. Compared with individuals without *CCR5-Δ32* or *CCR2-64I*, individuals with one or two copies of *CCR2-64I* had a 58% lower risk of AIDS during the first 4 years after seroconversion (RH 0.42, 95% CI 0.23,

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0.76), a 19% lower risk during the subsequent 4 years (RH 0.81, 95% CI 0.59, 1.12), and no significant protection thereafter.

Conclusion: The protection against AIDS provided by *CCR5-Δ32* is continuous during the course of infection. In contrast, the protection provided by *CCR2-64I* is greatest early in the course of infection.

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Introduction

HIV-1-infected individuals who carry *CCR5-Δ32* or *CCR2-64I* chemokine receptor gene polymorphisms progress significantly more slowly to clinical AIDS and death. Many studies have investigated these polymorphisms in individual cohorts [1–23]. A recent meta-analysis of these cohorts found that in the absence of potent antiretroviral therapy, both *CCR5-Δ32* and *CCR2-64I* carriers progressed to AIDS at a 25% slower rate than individuals who lacked either of these protective alleles [24]. They also progressed more slowly to death, by approximately 35% and 25%, respectively.

CCR5-Δ32 is a deletion mutation that renders the CCR5 cell surface receptor non-functional. CCR5 is the major co-receptor for the R5 HIV-1 variants (non-syncytium-inducing strains) that dominate in early infection. The deficit of HIV-1 co-receptors appears to impede viral replication *in vitro* [14,25,26]; and circulating levels of HIV-1 RNA in serum or plasma are lower during early chronic infection among *CCR5-Δ32* heterozygotes than in individuals with wild-type CCR5 [7,24]. In contrast, the *CCR2-64I* single nucleotide polymorphism codes for a conservative substitution and a mechanism for its effect remains to be elucidated. *CCR2-64I* is quite common in individuals of African and European descent, suggesting that it is a relatively old polymorphism that has been conserved over time [18]. In our population, the *CCR2-64I* allele occurs with a frequency of 0.10 and 0.14 in individuals of European and African descent, respectively. The *CCR5-Δ32* allele has a frequency of 0.09 in individuals of European descent.

Most studies have assumed that these gene variants restrict the rate of HIV-1 disease progression by a constant factor over time. However, Meyer *et al.* [7] reported that the survival benefit for *CCR5-Δ32* was limited to the first 7 years after seroconversion among subjects in the SEROCO cohort. A time-restricted effect was also observed in the Copenhagen seroprevalent cohort [23]. In the Copenhagen cohort, *CCR5-Δ32* carriers actually had poorer survival after the onset of AIDS [27]. Time-dependency was also reported for a CCR5 promoter polymorphism; disease progression was faster for *CCR5-P1* homozygotes, but only for the first 5 years after seroconversion [28].

Characterizing the temporal restriction of a gene's effect may shed light on its underlying biological mechanisms. However, the 'time-averaged' effects of these chemokine genes are relatively subtle, and individual HIV-1 cohort studies have limited power to dissect variation in the effects of these genes over time. In this paper, we analyse time-trends in the effects of the *CCR5-Δ32* and *CCR2-64I* polymorphisms using the largest available database, the International Meta-Analysis of HIV Host Genetics [24]. By pooling information from many studies, we increased our ability to characterize temporal patterns.

Methods

Data were contributed by 19 groups of investigators from the United States, Europe, and Australia [24]. Only cohorts of HIV-1 seroconverters were included in this analysis, provided that the subjects had been genotyped for both *CCR5-Δ32* and *CCR2-64I* and prospectively followed from seroconversion to AIDS (1987 definition) and death. These cohorts have typically enrolled and genotyped consecutive HIV-1 seroconverters, and thus there is no strong selection bias in favor of slow or rapid progressors.

Because time-averaged results between seroconverter cohorts were not significantly heterogeneous in the meta-analysis, we studied all of the seroconverters who had been included in that report [24], plus 18 additional individuals from five cohorts who were ineligible to be in the previous analysis because they belonged to subgroups with fewer than 20 individuals. We also included 80 additional individuals from the Multicenter Hemophilia Cohort Study who did not previously have genotype results. As in the previous analysis, we censored follow-up after 1 January 1996, to minimize the effects of potent antiretroviral therapy. In all, these participants contributed an additional 846.3 person-years of follow-up to AIDS, 38 AIDS events, 889.7 person-years of follow-up to death, and 43 deaths to the analysis. In total, we analysed 13 514.5 person-years

of follow-up, 619 AIDS events and 535 deaths (Appendix 1).

The CCR5 and CCR2 chemokine receptor genes are tightly linked on chromosome 3p21–22. Therefore, we analysed compound *CCR5-CCR2* genotypes to compare individuals who carried *CCR5-Δ32* or *CCR2-64I* with individuals who were homozygous for the wild-type allele of both genes (compound wild-type homozygotes). *CCR5-Δ32* homozygotes are highly resistant to infection and none were included in our analysis. *CCR5-Δ32* is restricted to individuals of European descent, whereas *CCR2-64I* is prevalent among individuals of both European and African ancestry. Therefore, our analysis of *CCR5-Δ32* was limited to individuals of European ancestry, whereas our analysis of *CCR2-64I* included individuals of either European or African ancestry. All models were adjusted for age at seroconversion, and stratified by cohort. *CCR2-64I* models were further stratified by racial ancestry. Neither CD4 T-cell count nor HIV-RNA load were included in our analyses, because these markers may be on the causal pathway through which these genetic variants affect the likelihood of AIDS or death.

The standard Cox proportional hazards model assumes that the effect of a covariate on the hazard rate for an event is constant over time. In our analysis, the covariate is compound *CCR5-CCR2* genotype and the outcome events are AIDS and death. This standard model provides a 'time-averaged' gene effect, and its statistical significance can be assessed using the likelihood ratio test. However, we hypothesized that *CCR5-Δ32* or *CCR2-64I* may not confer a constant protective effect over time, but instead may provide more protection early in the course of infection. To characterize potential time-variation in the effects of *CCR5-Δ32* and *CCR2-64I*, we relaxed the proportional hazards assumption using several prespecified models of gene-by-time interactions. These interaction models were based on categorical, cubic polynomial, and cubic spline functions of time (technical details are presented in Appendix 2).

For our primary analyses, *a priori* we tested the significance of categorical gene-by-time interactions based on 4-year time intervals (0–4, 4–8, 8–12, and 12+ years since seroconversion). However, when the number of events, or follow-up time, was sparse, we merged the first or last time intervals, respectively, with immediately adjacent intervals. In addition, previous studies reported that the protective effect of *CCR5-Δ32* is substantially diminished 7 years after infection [7]. Therefore, we also fitted a categorical gene-by-time interaction model with a single cutpoint at 7 years. Finally, in an exploratory analysis, we examined models that allow the relative hazard (RH) to vary continuously over time using cubic polynomial or cubic regression splines. However, for formal hypothesis tests, we relied on the categorical models, because these provide a limited number of easily interpretable parameters that characterize the RH over the entire time course of the disease.

We also fitted categorical gene-by-time interaction models to study survival after diagnosis of AIDS. For this period, we fitted a time-invariant model and a categorical interaction model with a single cutpoint 2 years after AIDS diagnosis.

We selected our final models using the following procedure. If none of the interaction models was statistically significant at the $\alpha = 0.05$ level, then the standard time-invariant model was selected. If one or more gene-by-time interaction model fit significantly better, then we selected the most parsimonious model using the Akaike Information Criterion (AIC) value (Appendix 2).

Finally, we contrasted the rate ratios for *CCR5-Δ32* and *CCR2-64I* using a direct approach based on a Poisson regression model of the log rates. This method tabulated the numbers of events and person-years of follow-up by cohort, genotype, and quadrennial periods since seroconversion; contrasting rate ratios were compared using a Wald test.

Results

Categorical gene-by-time interaction models

CCR5-Δ32 heterozygotes progressed significantly more slowly to AIDS than compound wild-type homozygotes (Wald chi-squared, $P = 0.003$) and death (Wald chi-squared, $P < 0.001$) based on the standard time-invariant Cox model. Moreover, no significant time-dependency was observed for the effect of *CCR5-Δ32* on the risk of AIDS or death, regardless of the model used (Table 1). *CCR5-Δ32* heterozygotes had approximately a 30% lower risk of AIDS and a 40% lower risk of death than compound wild-type homozygotes. None of the time-trends were statistically significant, although a trend towards subtle time-dependency was observed for the model with a single cutpoint at 7 years (Table 1), consistent with the original observation in the SEROCO cohort.

The *CCR5-Δ32* effect showed significant time-dependency when the period from AIDS diagnosis to death was isolated (Table 1). Compared with compound wild-type homozygotes, *CCR5-Δ32* heterozygotes had a statistically significant 43% lower risk of death during the 2 years after an AIDS diagnosis, but no significant protection thereafter.

Table 1. Categorical gene-by-time interactions for *CCR5-Δ32*.

Time from seroconversion (years)	Relative hazard	95% CI	−2 Log likelihood	DF	AIC	*P value
CCR5-Δ32 Seroconversion to AIDS						
TC	0.69	(0.54, 0.88)	4468.184	2	4472.184	NA
0–4	0.67	(0.39, 1.13)	4464.377	5	4474.377	0.28
⩾ 4–8	0.78	(0.56, 1.08)				
⩾ 8–12	0.63	(0.37, 1.08)				
⩾ 12	0.19	(0.04, 0.93)				
0–4	0.67	(0.39, 1.13)	4466.671	4	4474.671	0.47
⩾ 4–8	0.78	(0.56, 1.08)				
⩾ 8	0.54	(0.32, 0.90)				
0–8	0.75	(0.57, 0.99)	4464.644	4	4472.644	0.17
⩾ 8–12	0.63	(0.37, 1.08)				
⩾ 12	0.19	(0.04, 0.93)				
0–8	0.75	(0.57, 0.99)	4466.938	3	4472.938	0.26
⩾ 8	0.54	(0.32, 0.90)				
0–7	0.59	(0.43, 0.82)	4465.781	3	4471.265	0.12
⩾ 7	0.88	(0.60, 1.29)				
CCR5-Δ32 Seroconversion to death						
TC	0.60	(0.45, 0.78)	3601.146	2	3605.146	NA
0–4	0.76	(0.36, 1.61)	3600.692	5	3610.692	0.93
⩾ 4–8	0.57	(0.39, 0.84)				
⩾ 8–12	0.60	(0.35, 1.03)				
⩾ 12	0.56	(0.24, 1.30)				
0–4	0.76	(0.36, 1.61)	3600.707	4	3608.707	0.8
⩾ 4–8	0.57	(0.39, 0.84)				
⩾ 8	0.59	(0.37, 0.93)				
0–8	0.60	(0.42, 0.85)	3601.126	4	3609.126	0.99
⩾ 8–12	0.60	(0.35, 1.03)				
⩾ 12	0.56	(0.24, 1.30)				
0–8	0.60	(0.42, 0.85)	3601.141	3	3607.141	0.94
⩾ 8	0.59	(0.37, 0.93)				
CCR5-Δ32 AIDS to death						
TC	0.72	(0.53, 0.98)	2455.330	2	2459.330	NA
0–2	0.57	(0.39, 0.84)	2450.258	3	2456.258	0.02
⩾ 2	1.26	(0.73, 2.17)				

AIC, Akaike Information Criterion; TC, time constant model; effect of gene proportional over time.
*P value for difference in −2 log likelihood chi-squared test compared with time-constant model.
All models include only subjects of European ancestry, and are stratified on cohort.

Carriers of *CCR2-64I* were also protected against AIDS (Wald chi-squared, $P = 0.032$) and death (Wald chi-squared, $P = 0.034$) based on the standard time-invariant Cox model. Significant time-dependency was observed for the effect of *CCR2-64I* on the risk of AIDS (Table 2). However, the time-dependency was not significant for death or the period from AIDS to death. Compared with compound wild-type homozygotes, individuals with the *CCR2-64I* allele (homozygotes and heterozygotes combined) had a 58% lower risk of AIDS in the first 4 years after seroconversion. This declined to a 19% lower risk of AIDS during the subsequent 4 years, and no significant protection thereafter. Contrasting the two protective polymorphisms using a Poisson regression model of the AIDS hazard rate by cohort, quadrennial time period, and genotype showed that *CCR2-64I* carriers had a significantly lower rate of AIDS than *CCR5-Δ32* heterozygotes during the first 4 years after seroconversion (Wald chi-squared, $P = 0.04$).

Smooth gene-by-time interaction models

Effect of CCR5-Δ32 on AIDS incubation period and survival

Candidate gene-by-time interaction models are summarized in Table 3. To obtain a smooth estimate of the *CCR5-Δ32* effect over time, we fitted a cubic polynomial gene-by-time interaction model and five cubic regression spline models with knots at time 3 and 6 years, 4 and 8 years, and 5, 6, and 7 years. The cubic polynomial model had a superior AIC value to any of the regression splines, but it did not fit significantly better than the time-invariant model. A closer examination of the time-specific RH of AIDS estimated by the cubic polynomial model demonstrated that *CCR5-Δ32* heterozygotes are significantly protected from AIDS from approximately 2 to 5 years post-seroconversion (Fig. 1a). The RH for this period vary from 0.55 (95% CI 0.30, 0.99) to 0.71 (95% CI 0.51, 0.99). Estimates of the *CCR5-Δ32* effect during the first 2 years post seroconversion are imprecise because of the

Table 2. Categorical gene-by-time interactions for *CCR2-64I*.

Time from seroconversion (years)	Relative hazard	95% CI	−2 Log likelihood	DF	AIC	*P value
CCR2-64I Seroconversion to AIDS						
TC	0.78	(0.62, 0.98)	4884.18	2	4888.18	NA
0–4	0.42	(0.23, 0.76)	4876.629	5	4886.629	0.06
⩾ 4–8	0.81	(0.59, 0.76)				
⩾ 8–12	1.12	(0.72, 1.73)				
⩾ 12	0.88	(0.27, 2.84)				
0–4	0.42	(0.23, 0.76)	4876.774	4	4884.774	0.02
⩾ 4–8	0.81	(0.59, 1.12)				
⩾ 8	1.09	(0.72, 1.64)				
0–8	0.68	(0.51, 0.90)	4880.679	4	4888.679	0.17
⩾ 8–12	1.12	(0.72, 1.73)				
⩾ 12	0.88	(0.27, 2.84)				
0–8	0.68	(0.51, 0.90)	4880.824	3	4886.824	0.07
⩾ 8	1.09	(0.72, 1.64)				
0–7	0.66	(0.49, 0.89)	4881.024	3	4887.024	0.08
⩾ 7	1.02	(0.71, 1.47)				
CCR2-64I Seroconversion to death						
TC	0.76	(0.59, 0.98)	3979.67	2	3983.67	NA
0–4	0.76	(0.40, 1.47)	3975.233	5	3985.233	0.22
⩾ 4–8	0.67	(0.47, 0.98)				
⩾ 8–12	1.08	(0.69, 1.69)				
⩾ 12	0.35	(0.10, 1.19)				
0–4	0.76	(0.40, 1.47)	3978.7	4	3986.7	0.62
⩾ 4–8	0.67	(0.47, 0.98)				
⩾ 8	0.89	(0.59, 1.36)				
0–8	0.69	(0.50, 0.96)	3975.339	4	3983.339	0.11
⩾ 8–12	1.08	(0.69, 1.69)				
⩾ 12	0.35	(0.10, 1.19)				
0–8	0.69	(0.50, 0.96)	3978.805	3	3984.805	0.35
⩾ 8	0.89	(0.59, 1.36)				
CCR2-64I AIDS to death						
TC	0.96	(0.72, 1.29)	2641.85	2	2645.85	NA
0–2	0.91	(0.66, 1.26)	2641.16	3	2647.16	0.25
⩾ 2	1.25	(0.64, 2.42)				

AIC, Akaike Information Criterion; TC, time constant model; effect of gene proportional over time.
*P value for difference in −2 log likelihood chi-squared test compared with time-constant model.
All models are stratified on cohort and ancestry.

paucity of AIDS cases among *CCR5-Δ32* heterozygotes in this time period (2 events).

Similarly, the effect of *CCR5-Δ32* on survival did not demonstrate significant variation over time when seven cubic regression spline models with knots at time 3 and 8 years, 3 and 9 years, 4 and 10 years, and 5, 7, 8, and 10 years were evaluated. However, the graph of the cubic polynomial model demonstrates a protective benefit for *CCR5-Δ32* from approximately 3.3 to 8.2 years post-seroconversion, and a modest but not significant protective effect thereafter (Fig. 1b). The RH for the former period ranged from 0.49 (95% CI 0.31, 0.77) to 0.69 (95% CI 0.48, 0.99), indicating a 30–50% reduction in the risk of death. Infrequent fatalities among *CCR5-Δ32* heterozygotes before 4 years post-seroconversion resulted in extremely wide 95% confidence intervals during this period of follow-up.

Effect of CCR2-64I on AIDS incubation period and survival

CCR2-64I showed a strong, time-restricted effect in both categorical and smooth gene-by-time interaction models. The models consistently indicated an early protective effect for this polymorphism. Cubic polynomial and cubic spline gene-by-time interaction models with knots at 3 and 6 years, 4 and 8 years, and 4, 5, 6, and 7 years were investigated. Although none of the smoothed gene-by-time models reached statistical significance at the $\alpha = 0.05$ level, the cubic polynomial model, and hazard spline model with knots at 4 and 8 years, performed better than the time-invariant model based on the AIC. *CCR2-64I* provided substantial protection from AIDS during the first 5 years after seroconversion. The risk of AIDS was reduced by approximately 80% in the second year post-seroconversion, 50–70% in the third year, 40–50% in the fourth

Table 3. Continuous gene-by-time interaction models for *CCR5-Δ32* and *CCR2-64I* effects on incubation period and survival.

Continuous models of time-dependent relative hazards				
Time-interaction form	AIC	−2 Log likelihood	DF	*P value
CCR5-Δ32 time to AIDS				
TC	4472.18	4468.184	2	NA
Cubic polynomial	4473.44	4463.435	5	0.19
Cubic regression splines				
Knot at 3 and 6 years	4476.48	4462.475	7	0.34
Knot at 4 and 8 years	4475.74	4461.735	7	0.26
Knot at 5 years	4474.51	4462.514	6	0.23
Knot at 6 years	4474.88	4462.883	6	0.26
Knot at 7 years	4475.22	4463.224	6	0.29
CCR5-Δ32 time to death				
TC	3605.15	3601.146	2	NA
Cubic polynomial	3607.77	3597.772	5	0.34
Cubic regression splines				
Knot at 3 and 8 years	3608.42	3594.416	7	0.24
Knot at 3 and 9 years	3609.03	3595.029	7	0.30
Knot at 4 and 10 years	3610.72	3596.722	7	0.49
Knot at 5 years	3609.44	3597.437	6	0.45
Knot at 7 years	3609.76	3597.759	6	0.50
Knot at 8 years	3609.77	3597.766	6	0.50
Knot at 10 years	3609.48	3597.476	6	0.45
CCR2-64I time to AIDS				
TC	4888.18	4884.180	2	NA
Cubic polynomial	4887.1	4877.099	5	0.07
Cubic regression splines				
Knot at 3 and 6 years	4889.55	4875.548	7	0.12
Knot at 4 and 8 years	4888.05	4874.049	7	0.07
Knot at 4 years	4888.24	4876.242	6	0.09
Knot at 5 years	4888.57	4876.567	6	0.11
Knot at 6 years	4888.88	4876.884	6	0.12
Knot at 7 years	4889.08	4877.084	6	0.13
CCR2-64I time to death				
TC	3983.67	3979.670	2	NA
Cubic polynomial	3986.55	3976.553	5	0.37
Cubic regression splines				
Knot at 3 and 9 years	3983.12	3969.121	7	0.06
Knot at 3 and 8 years	3983.01	3969.005	7	0.06
Knot at 4 and 10 years	3984.43	3970.433	7	0.10
Knot at 5 years	3984.02	3972.023	6	0.11
Knot at 7 years	3982.86	3970.857	6	0.07
Knot at 8 years	3982.38	3970.384	6	0.05
Knot at 10 years	3984.36	3972.361	6	0.12

AIC, Akaike Information Criterion; TC, time constant model; effect of gene proportional over time.
*P value for difference in −2 log likelihood chi-squared test compared with time-constant model.
Models for *CCR5-Δ32* include only subjects of European ancestry, and are stratified on cohort. Models for *CCR2-64I* are stratified on cohort and ancestry.

year, and 30–40% in the fifth year post-seroconversion. (Fig. 1c).

CCR2-64I also had a time-restricted effect on survival. Smoothed estimates were calculated using cubic polynomial and cubic spline gene-by-time interaction models with knots at 3 and 8 years, 3 and 9 years, 4 and 10 years, and 5, 7, 8, and 10 years. A cubic spline with a knot at 8 years performed significantly better than the time-invariant model, and had the best AIC value (Fig. 1d). The effect of *CCR2-64I* on survival was seen later in the disease course than the effect on AIDS risk. Early estimates are imprecise because of the paucity of deaths among *CCR2-64I* carriers before 4 years post-seroconversion, and estimates beyond 10 years are also imprecise.

Discussion

It has frequently been assumed that the inherited resistance against progression to AIDS and death conferred by the *CCR5-Δ32* and *CCR2-64I* genetic

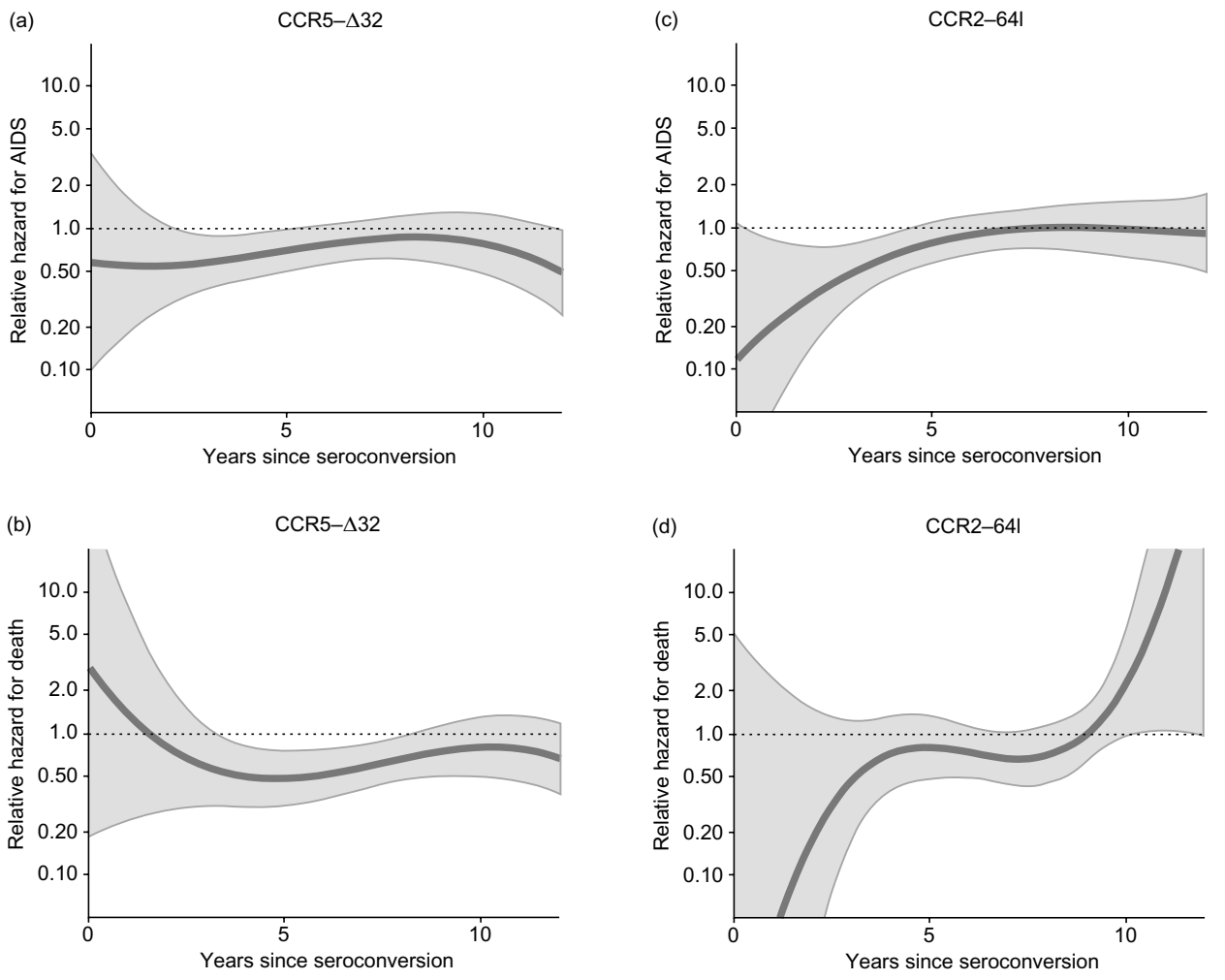

Fig. 1. Cubic polynomials describing the relative hazards of AIDS and death in *CCR5-Δ32* and *CCR2-64I* carriers. (a) Cubic polynomial describing the relative hazard of AIDS in *CCR5-Δ32* carriers relative to *CCR5* and *CCR2* compound wild-type homozygotes. *CCR5-Δ32* carriers have a lower risk of AIDS from 2–5 years after seroconversion; qualitatively, the relative hazard is roughly constant over time. (b) Cubic polynomial describing the relative hazard of death in *CCR5-Δ32* carriers. *CCR5-Δ32* carriers have significant protection from death from approximately 3–8 years after seroconversion, and the relative hazard is roughly constant over time. (c) Cubic polynomial describing the relative hazard of AIDS in *CCR2-64I* carriers relative to *CCR5* and *CCR2* compound wild-type homozygotes. *CCR2-64I* carriers have a lower risk of AIDS during the first 4 years after seroconversion. (d) Relative hazard of death in *CCR2-64I* carriers modeled using a cubic regression spline with a knot at 8 years after seroconversion. *CCR2-64I* carriers have a survival advantage that diminishes over time. Estimates for the early years are imprecise because relatively few *CCR2-64I* carriers died during these years. Estimates for later years are also imprecise.

polymorphisms is constant over the course of the disease. In reality, the relationships may be more complex. The large amount of data available from the International Meta-Analysis of HIV Host Genetics [24] allowed us to fit flexible gene-by-time interaction models for the effects of *CCR5-Δ32* and *CCR2-64I*. We implemented a vigorous and systematic approach to model selection to avoid over-interpreting results. The findings of time-dependence were largely consistent, regardless of the modeling approach being employed. The models that best characterize the effects of these polymorphisms are presented in Table 4.

We observed no significant time-dependency for the effect of *CCR5-Δ32* on progression from seroconversion to AIDS or from seroconversion to death. A previous report found a suggestion of time-dependency in the *CCR5-Δ32* effect on progression to AIDS, but it did not reach statistical significance [7]. Our data suggest that any temporal effects of the *CCR5-Δ32* polymorphism on progression to AIDS are likely to be modest. However, we did observe significant time-dependency for the period from AIDS diagnosis to death. *CCR5-Δ32* heterozygotes remain protected from death for approximately 2 years after an AIDS diagnosis, but thereafter have no protection, and possibly an increased risk. By 2 years after AIDS, the surviving group may be enriched with individuals

Table 4. Final models for *CCR5-Δ32* and *CCR2-64I* effects on incubation period and survival.

Gene	Model	Time	Relative hazard	95% CI	*P value
CCR5-Δ32	Seroconversion to AIDS	TC	0.69	(0.54, 0.88)	
	Seroconversion to death	TC	0.60	(0.45, 0.78)	
	AIDS to death	0–2 years	0.57	(0.39, 0.84)	0.02
		⩾ 2 years	1.26	(0.73, 2.17)	
CCR2-64I	Seroconversion to AIDS	0–4 years	0.42	(0.23, 0.76)	0.02
		⩾ 4–8 years	0.81	(0.59, 1.12)	
		⩾ 8 years	1.09	(0.72, 1.64)	
	Seroconversion to death	TC	0.76	(0.59, 0.98)	
	AIDS to death	TC	0.96	(0.72, 1.29)	

TC, time constant model; effect of gene proportional over time.
*P value for difference in −2 log likelihood chi-squared test compared with time-constant model.
Models for *CCR5-Δ32* include only subjects of European ancestry, and are stratified on cohort.
Models for *CCR2-64I* are stratified on cohort and ancestry.

whose circulating virus can use the CXCR4 co-receptor [26]. However, X4 viruses often emerge before AIDS [29,30]; and even after they emerge, X4 viruses may not be the only strains circulating in an individual [1]. Therefore, this explanation for the waning *CCR5-Δ32* effect requires further study.

In contrast to *CCR5-Δ32*, the effect of the *CCR2-64I* polymorphism on progression to AIDS varied over time. During the first 4 years after seroconversion, the protective effect of this variant is larger than previously recognized; moreover, carriers of *CCR2-64I* are significantly more protected from an AIDS event than carriers of *CCR5-Δ32* during this period. Our previous meta-analysis of this database showed that in a 'time-averaged' sense, *CCR2-64I* is associated with a 24% lower risk of AIDS. However, using time-dependent models, we found *CCR2-64I* carriers had approximately a 60% lower risk of AIDS in the first 4 years after seroconversion, a 20% lower risk of AIDS during the subsequent 4 years, and no significant protection thereafter.

A mechanism for the *CCR2-64I* effect remains to be elucidated. As CCR2 is a minor co-receptor for HIV-1, it is unlikely that the profound reduction in the risk of AIDS that we observed in the first 4 years after seroconversion directly reflects the role of CCR2 as a viral co-receptor. Instead, a mechanism involving CCR5 or CXCR4, the major HIV-1 co-receptors, is more likely. It has been proposed that the *CCR2-64I* variant may be in linkage disequilibrium with a functional mutation in CCR5 that travels on the same haplotype [5], or that the protein produced by the *CCR2-64I* variant may interact with CCR5 or CXCR4 [31,32] to decrease the expression of these co-receptors. Although the 64I variant has not been associated with altered baseline expression rates of CCR5 [31,33], it has recently been reported that the CD4 T-lymphocytes in individuals who carry the *CCR2-64I* variant may be slower to re-express CCR5 after internalization, which could result in less CCR5 available on the cell surface [34]. Other studies have shown that *CCR2-64I* carriers develop X4 HIV-1 strains more rapidly than wild-type individuals [35–37], and that the *CCR2-64I* protection is lost after X4 strains emerge [36]. Putting these data together with our findings of an early effect for *CCR2-64I*, one could speculate that the allele may provide early protection through an interaction with CCR5, but that this protection is lost after X4 strains emerge in these patients. Further epidemiological studies of *CCR2-64I* and viral phenotype in early chronic infection are needed to examine this hypothesis.

The potential limitations of our study must be considered. First, not all subjects in the cohorts were genotyped. Our results might be subject to bias if the clinical course and genotype frequency differ in those who were not genotyped. However, the most plausible direction of this potential bias strengthens our main conclusions because rapid progressors may be less likely to have samples available for genotyping (because of shorter survival) and more likely to be wild-type for the *CCR2-64I* and *CCR5-Δ32* alleles (because of the observed protective effects of the mutant alleles). Second, although our statistical methods are sensitive and our database is the largest available, the genetic effects are subtle. As a consequence, our estimates are somewhat imprecise, as reflected by rather broad confidence intervals. Finally, survival in some cohorts might be better than others because of differences in access to healthcare or socioeconomic status. As allele frequencies also differ by cohort (Appendix 1), one might be concerned that our results could be affected by confounding. Fortunately, the cohort-stratified survival analysis we used protects us from this potential bias: it controls very closely for the effects of cohort on disease progression by allowing each cohort to have a different baseline hazard function.

The course of HIV-1 infection is governed by multiple genetic factors, which may operate at different time intervals. Our finding that the effect of *CCR2-64I* is limited to early infection may shed light on the mechanism by which this allele improves prognosis in HIV-1 infection, which is heretofore unexplained. It also suggests that similar analyses for other candidate genetic polymorphisms that are being proposed as regulators of the rate of HIV-1 disease progression may be of interest. Large-scale analyses, similar to this pooled analysis of individual patient data, would be required to probe such time-dependent associations.

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Appendix 1

The following table describes the cohorts included in this pooled analysis of HIV-1 seroconverters. Two subjects of African descent in the AIDS Link to the Intravenous Experience cohort who had been included in the previous meta-analysis [24] were excluded here because they were missing *CCR5-Δ32* genotype data. The analysis excludes seroprevalent cohorts because the dates of seroconversion are unknown, and as a consequence, it would be difficult to interpret time trends in the effects of a gene.

Cohort	N	Person-years follow-up to AIDS (1987 definition)	AIDS events (1987 definition)	Person-years follow-up to death	Death events	*CCR5-Δ32* allele frequency (%)	*CCR2-64I* allele frequency (%)
Cohorts of European descent							
Multicenter AIDS Cohort Study	405	2633.045	183	2871.104	144	8.8	10.0
Amsterdam Cohort of Homosexual Men	122	677.115	51	765.117	48	7.4	9.4
Amsterdam Cohort Among IV Drug Users	63	274.854	8	285.314	11	9.5	8.7
San Francisco City Clinic Cohort	22	161.496	4	172.189	3	15.9	13.6
SEROCO	355	2199.113	113	2381.19	77	8.7	8.6
San Francisco Men's Health Study	36	273.822	17	291.49	11	13.2	6.6
Swiss HIV Cohort Study	265	1795.158	36	1859.437	21	8.1	10.6
Multicenter Hemophilia Cohort Study	314	3093.661	137	3283.551	133	7.7	10.1
AIDS Link to the Intravenous Experience	8	33.4	0	33.4	0	12.5	12.5
District of Columbia Gay Cohort	45	335.11	26	364.451	45	11.1	6.5
Cohorts of African descent							
Multicenter AIDS Cohort Study	40	223.102	10	231.874	8	2.3	16.2
San Francisco City Clinic Cohort	3	17.958	0	17.958	0	16.7	16.7
SEROCO	7	40.081	3	45.843	2	0.0	0.0
San Francisco Men's Health Study	1	6.734	1	8.225	1	0.0	0.0
Swiss HIV Cohort Study	5	16.142	1	16.469	1	0.0	10.0
Multicenter Hemophilia Cohort Study	25	268.745	10	280.651	6	3.8	16.0
AIDS Link to the Intravenous Experience	132	572.781	17	595.245	22	0.4	13.1
District of Columbia Gay Cohort	2	8.736	2	10.973	2	0.0	50.0

Appendix 2

Let t be the years-since-seroconversion, I{.} be the indicator function equal to 1 if a logical condition in brackets is true, 0 otherwise, and $(x)_+ = \max(0,x)$ be the 'truncation' function. Let $GENE$ = '+' if wild-type, '−' if variant, AGE be the age-at-seroconversion, $ANCESTRY = 1$ if African, 0 if European and $h_c{}^0(t)$ be an unspecified baseline hazard function that is specific for cohort c. The baseline hazard function specifies the event rate (of AIDS or death) that will occur in an infinitesimal time interval $[t,t+\Delta)$ among susceptible individuals in cohort c who are still at risk at time t and who have baseline covariate values for $GENE$, AGE, and $ANCESTRY$. (Models for *CCR2-64I* include $ANCESTRY$ whereas models for *CCR5-Δ32* do not.)

The standard time-invariant Cox proportional hazards model is

$$\log h(t|GENE,\ AGE) = \log h_c{}^0(t) + \alpha AGE + \beta I\{GENE = \text{'}-\text{'}\}.$$

This model implies that the log relative hazard (log $RH(t)$) contrasting individuals with the variant allele with individuals with the wild-type allele is constant over time, $\log RH(t) = \beta$.

An extended Cox model with a categorical time interaction at 5 years is

$$\log h(t|GENE,\ AGE) = \log h_c{}^0(t) + \alpha AGE + \beta I\{GENE = \text{'}-\text{'}\} + \delta \mathrm{I}\{GENE = \text{'}-\text{'}\}(t-5)_+.$$

This model implies that

$$\log RH(t) = \begin{cases} \beta \text{ if } t \leqslant 5 \\ \beta + \delta \text{ if } t > 5 \end{cases}$$

An extended Cox model with a cubic polynomial time interaction is

$$\log h(t|GENE,\ AGE) = \log h_c{}^0(t) + \alpha AGE + \beta I\{GENE = \text{'}-\text{'}\} + \gamma_1 I\{GENE = \text{'}-\text{'}\}t + \gamma_2 I\{GENE = \text{'}-\text{'}\}t^2 + \gamma_3 I\{GENE = \text{'}-\text{'}\}t^3$$

which implies that

$$\log RH(t) = \beta + \gamma_1 t + \gamma_2 t^2 + \gamma_3 t^3.$$

The following equation describes an extended Cox model with a gene-by-time interaction specified using a cubic regression spline with knots at 4 and 8 years:

$$\log h(t|GENE,\ AGE) = \log h_c{}^0(t) + \alpha AGE + \beta \mathrm{I}\{GENE = \text{'}-\text{'}\} + \gamma_1 I\{GENE = \text{'}-\text{'}\}t + \gamma_2 I\{GENE = \text{'}-\text{'}\}t^2 + \gamma_3 I\{GENE = \text{'}-\text{'}\}t^3 + \gamma_4 I\{GENE = \text{'}-\text{'}\}(t-4)_+{}^3 + \gamma_5 I\{GENE = \text{'}-\text{'}\}(t-8)_+{}^3.$$

For this model, $\log RH(t)$ is a cubic regression spline with knots at 4 and 8 years:

$$\log RH(t) = \beta + \gamma_1 t + \gamma_2 t^2 + \gamma_3 t^3 + \gamma_4 (t-4)_+{}^3 + \gamma_5 (t-8)_+{}^3$$

This equation specifies three cubic polynomials for the intervals (0,4], (4,8], and (8,t_{max}); the pieces join together smoothly so that the function and its first two derivatives are continuous.

Each of the extended Cox models contains the time-invariant model as a special case; statistical significance of parameters describing gene-by-time interactions is assessed using the standard likelihood ratio test. In the models described above, there are 1, 3, and 5 degrees of freedom, respectively, for interaction.

In our analyses of *CCR5-Δ32* and *CCR2-64I*, we fitted several categorical- and cubic-spline-based models of gene-by-time interaction in order to determine which model provides the best description of the genes' effects. Model selection was performed using a two-step procedure. First, we ruled out interaction models that were not statistically significant at the $\alpha = 0.05$ level using the likelihood ratio test. Second, if more than one model was significant, we selected the model with the lowest value of the AIC [38]. The AIC value is defined as −2 times the log likelihood ratio of the model, plus two times the number of degrees of freedom. AIC is widely used to balance goodness-of-fit and parsimony.